Effect of Vitamins E, B_{12} and Folacin on CCL_4 Toxicity and Protein Utilization in Rats.* (18828)

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Popper, Koch-Weser, and Szento(1) reported that vit. B_{12} protected against carbon tetrachloride damage to livers in rats maintained on an unspecified diet. Previously Hove(2) had shown that vit. E protected rats against death due to CCl_4 when the animals were reared on a 10% casein diet deficient in vit. E. Since vit. B_{12} and folacin were not added to this diet, it became of interest to determine the influence of these vitamins on the protective action of vit. E against acute CCl_4 poisoning.

Experimental. The diets contained a 10% protein level as furnished either by water-washed commercial casein or by commercial solvent extracted soybean meal. The remainder of the diet consisted of salt mixture 4%, lard 9%, cod liver oil 1%, and sucrose to 100%. Pure vitamins were added in amounts such that each gram of diet contained: thiamine, riboflavin and pyridoxine, 5 μg each; calcium pantothenate, 25 μg; niacin 40 μg; choline chloride, 2 mg; i-inositol, 0.2 mg; and 2-methyl 1-4 naphthoquinone, 2 μg. These diets were supplemented as indicated with DL-α-tocopheryl acetate, vitamin B_{12} as furnished by a Fuller's earth concentrate containing 120 μg/g (Merck), and folacin as Folvite (Lederle).

Male rats of the Sprague-Dawley strain were placed on the diets and supplements, *ad libitum*, at weaning (40 to 50 g) and maintained for periods of 4 to 12 weeks in individual cages. Weekly body weight records were kept, and food consumption was measured for the rats on the soybean meal diets. Protein efficiency ratios were calculated for 4-week periods by dividing the total gain in body weight by the total protein ingested. Presumably this represents the efficiency with which the animals utilize dietary protein for growth.

The sensitivity of the rats to CCl_4 was determined by injecting this substance intraperitoneally at the levels indicated, and noting the times at which deaths occurred. Observations were made for 1 week but, as noted previously(2), most deaths came within 48 hours and therefore the data have been expressed as the number and per cent survivals at this time. The single CCl_4 injections were made after the rats had been on the various diets for periods ranging from 4 to 12 weeks. The age of the animals at the time of injection appeared not to influence the results and this factor has been ignored in combining the data. As determined in preliminary studies, both the level and duration of the tocopherol supplementation had a distinct bearing on the results. It was noted that the 0.01% dietary tocopherol level had to be fed for at least 2 weeks prior to poisoning to obtain protection. However, a single dose of tocopherol, if high enough, afforded protection; 25 mg given orally to 12 rats 2 days prior to the CCl_4 permitted survival of 8 (67%), but 5 mg so administered was without benefit. Therefore, all supplemented diets were fed for at least 4 weeks in the presently reported experiments.

Results. Protein utilization of rats on the soybean meal basal diet was significantly improved by the α-tocopheryl acetate supplement (Table I). A similar effect, noted with the casein diet (Table II), was reported pre-

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1. Popper, H., Koch-Weser, D., and Szento, P. B., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 688.

2. Hove, E. L., *Arch. Biochem.*, 1948, v17, 467.

TABLE I. Protein Utilization and CCl_4 Sensitivity of Rats on 10% Soybean Protein Diet.

Additions to 100 g of soybean diet	No. of rats	Wt gain 4 wk, g	Protein efficiency ratios		Survivals 48 hr after levels of CCl_4/kg, of: 2 ml	1.5 ml	1 ml
					%	%	%
None	33	26	1.75	.064*	2/20 (10)	3/7 (43)	3/6 (50)
α-tocopheryl acetate, .01 g	23	34	2.29	.046	15/18 (83)	—	5/5(100)
Vitamin B_{12}, 3 μg	16	31	2.03	.081	1/9 (11)	—	4/7 (57)
Vit. B_{12} and toc. acet.	11	27	1.88	.073	6/6 (100)	—	5/5(100)
Folacin, 1 mg	7	18	1.62	.105	—	1/7 (14)	—
Folacin and vit. B_{12}	11	37	2.47	.062	0/5 (0)	2/6 (33)	—
Folacin, vit. B_{12} and tocopheryl acet.	11	38	2.59	.076	3/5 (60)	6/6(100)	—

* Std error of mean.

TABLE II. Growth and CCl_4 Sensitivity of Rats on a 10% Casein Diet.

Additions to 100 g of casein diet	No. of rats	Wt gain 4 wk, g	Survivals 48 hr after levels of CCl_4/kg, as follows: 2 ml	1.5 ml	1 ml
			%	%	%
None	72	34	6/28(21)	4/17 (24)	13/27 (48)
α-tocopheryl acetate, .01 g	70	40	24/29(83)	12/14 (85)	25/27 (93)
Vit. B_{12}, 3 μg	21	44	12/16(75)	—	4/5 (80)
,, and toc. acet.	20	53	13/15(87)	—	5/5 (100)
Folacin, 1 mg	10	26	—	2/10 (20)	—
,, and toc. acet.	10	48	—	10/10(100)	—
Aminopterin, 70 μg	14	25	—	2/14 (14)	—
,, and toc. acet.	19	37	—	16/19 (85)	—
L-cystine, .1 g	11	52	0/5 (0)	—	6/6 (100)
,, .3 g	10	51	—	—	9/10 (90)
,, 1 g	16	68	7/10 (70)	—	5/6 (85)
Cystine, .3, and toc. acet.	8	74	—	—	7/9 (78)
DL-methionine, .1 g	6	56	—	—	5/6 (83)
Methionine and toc. acet.	6	62	—	—	5/6 (83)
Theophylline, .1 g	4	33	—	—	4/4 (100)
,, and toc. acet.	4	42	—	—	4/4 (100)
Creatine, .5 g	8	39	0/8 (0)	—	—
,, and toc. acet.	8	53	7/8 (88)	—	—
Guanidoacetic acid, .5 g	8	29	4/8 (50)	—	—
,, and toc. acet.	8	31	2/8 (25)	—	—

viously(3-4). Supplements of vit. B_{12} improved protein utilization on the vitamin E-low soybean diet, but folacin supplements were ineffective. On this diet the combined supplement of vit. B_{12} and folacin resulted in a protein efficiency value that was significantly higher than that given by vit. B_{12} alone. The addition of tocopherol together with the vit. B_{12} and folacin resulted in a still higher value.

Excellent protection against the acute CCl_4 toxicity in the rats was conferred by supplementation of either diet with vit. E (Tables I and II). Dietary vit. B_{12} protected rats on the casein basal diet against the lethal effect of CCl_4, but on the soybean meal basal diet this vitamin, at the level fed, was completely ineffective, even when combined with folacin. Folacin supplements alone were without benefit to rats on either diet. The major treatment effects (Table I) may be summarized as follows: On the soybean meal basal diet, the supplements of vit. B_{12}, folacin, or both, allowed survival of 8 out of 34 rats (23%). On the same diets to which tocopherol was added, 40 out of 45 rats (89%) survived. Similarly, on the casein basal diet (Table II) supplemented with

3. Hove, E. L., and Harris, P. L., *J. Nutrition,* 1947, v34, 571.

4. Moore, T., *Annals N. Y. Acad. Science,* 1949, v52, 206.

tocopherol, 113 out of the 129 rats (88%) survived, and on the casein diets to which vitamin B_{12} was added, 16 of the 21 rats (76%) lived past the 48-hour point.

These data indicate that the protective effect of the vit. B_{12} against acute CCl_4 toxicity depends upon the nature of the basal diet, since it was positive on the casein diet but negative on the soybean diet, at the levels fed. However, an explanation of this discrepancy may be found on considering the dosage levels. A dietary vit. B_{12} level of 3 μg/100 g diet has been considered fully adequate for the rat, and indeed this level did significantly improve the protein utilization in rats on the soybean diet (Table I). The requirement of vit. B_{12} for protection against CCl_4 may be higher than the level used. To test this, an experiment was set up in which vit. B_{12} was added at the levels of 0, 3, and 30 μg/100 g of each basal diet. After injecting CCl_4 (2 ml/kg body weight) into rats reared on these diets, it became evident that the higher (30 μg) level of vit. B_{12} protected rats on the soybean diet, but that the "normal" (3 μg) level did not; the values for survival were 6 out of 8 rats (75%) and 1 out of 5 rats (20%), respectively. Survival in the control group was also 1 out of 5. Survival of rats on the casein diets were 80% on the 3 μg level, and 66% of the rats on the 30 μg level of vit. B_{12}. Growth of the rats was not increased by the higher level of this vitamin as compared with the lower level.

It was previously noted(2) that methionine and theophylline added at a 0.1% level to the casein diet deficient in vit. E gave excellent protection against CCl_4. This is confirmed by data in Table II. Dietary creatine was without benefit, while dietary guanidoacetic acid appeared from limited data to suppress both growth and the protective action of vit. E.

L-Cystine gave fairly good protection against acute CCl_4 toxicity (Table II). Preliminary results showed that the partial protection given by the lower levels of cystine and tocopherol was not improved by combining these supplements (Fig. 1). Experiments are currently underway to determine the possible relation of vit. B_{12} and folacin to the protective effect of the sulfur amino acids.

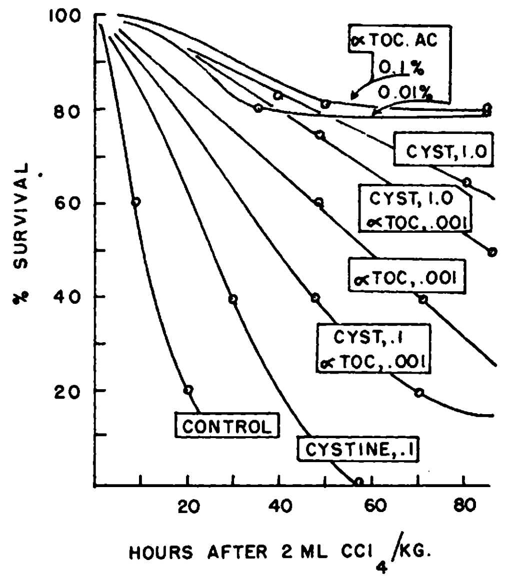

FIG. 1. Influence of various percentage levels of cystine and DL α-tocopheryl acetate added to the 10% casein diet, on susceptibility of rats to acute CCl_4 toxicity. Five to 6 male rats per group on the diets and supplements for 6 weeks from weaning.

Discussion. An interrelation of vit. E and vit. B_{12} has been noted in that they were mutually replaceable when using as criteria either the increased protein utilization or the protection of rats on 10% protein diets against the lethal effects of CCl_4. Apparently the rat has a much higher vit. B_{12} requirement for protection against CCl_4 than it does for growth. On the soybean basal diet a good protein utilization response was obtained with vit. B_{12} added at a level of 3 μg/100 g, but this had to be increased to 30 μg/100 g to get protection against CCl_4. Folacin supplements alone did not have an influence on protein utilization, but the data suggest that this vitamin influences the results obtained with vit. B_{12} and must be added to get maximum effect, as has been noted previously by Schaefer and co-workers(5) in work relating these vitamins to transmethylation and the choline requirement.

5. Schaefer, A. E., Salmon, W. D., Strength, D. R., and Copeland, D. H., *J. Nutrition,* 1950, v40, 95.

Presumably, the casein basal diet contained enough vit. B_{12} as an impurity in the casein so that the additional supplement of 3 μg/100 g was sufficient for protection against the CCl_4. Growth of rats on the casein basal diet was not significantly improved by supplementation with vit. B_{12} alone. The apparent growth improvement seen in the data in Table II was not nearly so evident when comparison was made with controls from the specific experiment. Dilution of these control values with large numbers of control values from other experiments was done to emphasize the lethal influence of CCl_4 since this was not affected by growth or body size of the rats.

Goyco(6) recently reported on the beneficial effect of vit. B_{12}, vit. E and methionine on utilization of yeast protein by rats. His results were similar in many respects to the data obtained with the soybean meal protein diet and herein reported.

Summary. Vit. E and vit. B_{12} can replace each other in promoting protein utilization and in protecting against acute carbon tetrachloride toxicity in young rats reared on 10% protein diets deficient in these factors. The vit. B_{12} requirement for protection against CCl_4 is considerably higher than for growth. Folacin supplements, alone, had no effect, but, in the presence of vit. B_{12} or vit. E, this factor markedly improved protein utilization and growth. L-Cystine protected against the acute toxicity but a synergism between cystine and vit. E was not noted.

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Inhibition of Influenza and Mumps Viruses in Tissue Culture by Basic Amino Acids.* (18829)

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Recent studies(1-4) have indicated that modification of the host-cell metabolism by a variety of substances may interfere with the growth of animal viruses in tissue culture. Emphasis has been placed on inhibition of viruses by metabolic antagonists such as methoxinine and malonic acid(1-3) but an excess of amino acids may also inhibit virus multiplication as exemplified by the effects of lysine and tryptophane on a neurotropic virus in mouse brain tissue cultures(1). In the present study inhibition of the growth of influenza and mumps viruses by arginine and other amino acids was observed. Investigations on the effects of these substances on the viruses and tissues themselves are reported.

Methods. Tissue cultures of minced chorioallantoic membrane of 10 to 12 day old chick embryos in balanced salt solution with glucose and sodium bicarbonate were prepared according to methods previously described(4-5). Allantoic passage strains of mumps and influenza (PR8 and Lee) were inoculated at dilutions of 10^{-2} into tissue cultures containing the substances to be tested. Cultures containing mumps and the PR8 strain of influenza were incubated at 35°C, those with the Lee strain at 31°C. Virus multiplication was measured by hemagglutination titrations with chicken erythrocytes

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